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Mechanism of α-Glycerophosphate Regulation of Acetyl-Coenzyme A Carboxylase of *Saccharomyces cerevisiae*

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The mechanism proposed for the activation of animal acetyl-coenzyme A (CoA) carboxylase by α-glycerophosphate, namely, the removal of inhibitory palmityl-CoA via glyceride synthesis, is not the only possible one in the yeast system because extracts exhibiting marked stimulation of acetyl-CoA carboxylase activity by α-glyerophosphate show a lack of acyl-CoA compounds.

Previous detailed data from this laboratory (12, 14, 15) have shown that fatty acid synthesis in extracts of *Saccharomyces cerevisiae*, strain LK2G12, can be stimulated by a number of intermediates of the glycolytic pathway and of the Krebs cycle. It was demonstrated that, as in animal systems (3, 13), the carboxylation of acetyl-coenzyme A (CoA) is the rate-limiting step (14) and that many of the same substances which stimulate fatty acid synthesis also stimulate the acetyl-CoA carboxylase (11, 12, 14, 15).

Citrate and isocitrate stimulation of the acetyl-CoA carboxylase from animal sources involves an allosteric modification that is associated with an increase in molecular weight of the enzyme (4, 9, 13). In contrast, Howard and Lowenstein (5, 6) ascribed stimulation of fatty acid synthesis by α-glycerophosphate to the relief of "end-product inhibition." Using rat liver preparations, they pointed out that palmityl-CoA inhibited acetyl-CoA carboxylase, and they postulated that α-glycerophosphate could stimulate this enzyme by combining with palmityl-CoA to form glycerides. We also have found that palmityl-CoA similarly inhibits fatty acid synthesis in extracts of *S. cerevisiae* (14, 15). The studies reported here were designed to test whether α-glycerophosphate stimulation of the acetyl-CoA carboxylase of this strain of yeast could be explained by the mechanism of relief of end-product inhibition, as has been proposed for the rat system.

MATERIALS AND METHODS

Information on the organism used in this study, *S. cerevisiae* strain LK2G12, its cultivation, harvesting, disintegration, and subsequent centrifugal fractionation was summarized in an earlier publication (15). The source of acetyl-CoA carboxylase was a 0 to 35% ammonium sulfate fraction of a dialyzed high-speed supernatant fluid. This fraction was stable for several months when stored at −70 C.

Proteins were determined by the method of Lowry et al. (7), with Prosol (bovine serum albumin) as the standard.

Acetyl-CoA carboxylase activity was followed by the incorporation of ^{14}C-bicarbonate according to the method of Martin and Vagelos (8), except that radioactivity was determined by scintillation counting after first drying the samples in vacuo, then dissolving each in 0.1 ml of water, and adding 10 ml of scintillation fluid (15).

Acyl-CoA L-α-glycerophosphate acyl transferase activity was measured by the incorporation of ^{14}C-α-glycerophosphate into lipids. For this assay, the enzyme preparations were incubated for 45 min at 30 C with uniformly labeled ^{14}C-α-glycerophosphate, palmityl-CoA, and appropriate cofactors. ^{14}C-glycerides were extracted by the procedure of Bligh and Dyer (1) for total lipids, and then counted for radioactivity.

Adenosine triphosphate (ATP) was purchased from the Boehringer-Mannheim Corp. (New York, N.Y.); enzyme-grade ammonium sulfate, from Mann Research Laboratories (New York, N.Y.); DL-α-glycerophosphate, palmityl-CoA, and acetyl-CoA, from the Sigma Chemical Co. (St. Louis, Mo.); and uniformly labeled ^{14}C-α-glycerophosphate, from International Chemical and Nuclear Corp. (City of Industry, Calif.). All other radioactive chemicals were obtained from the New England Nuclear Corp. (Boston, Mass.).

RESULTS

The results shown in Fig. 1 indicate that palmityl-CoA inhibits the acetyl-CoA carboxylase

of this strain of yeast with half-maximal inhibition occurring at about 9×10^{-6} M palmityl-CoA. This value is consistent with 3.2×10^{-5} M presented earlier (15) for half-maximal inhibition by palmityl-CoA of fatty acid synthesis in cruder preparations.

When we used inhibitory concentrations of palmityl-CoA, we found that both α-glycerophosphate and citrate alleviated inhibition by palmityl-CoA (Table 1). At the lower concentrations, α-glycerophosphate or citrate overcame the inhibition, and at higher concentrations further stimulation occurred—although the stimulation was never as great as it was in the absence of palmityl-CoA.

To determine whether the observed stimulation of acetyl-CoA carboxylase by α-glycerophosphate could be caused by relief of end-product inhibition by glyceride synthesis, the enzyme preparation was tested for the presence of acyl-CoA L-α-glycerophosphate acyl transferase. Clearly, if this enzyme were absent from the preparation, the proposed mechanism would be untenable. We found that (Fig. 2) this enzyme was present in the ammonium sulfate fraction used. However, we also observed that, in the absence of added palmityl-CoA, there was no incorporation of α-glycerophosphate into glycerides. The strict dependence of this reaction on added palmityl-CoA, even in amounts considerably less than required to inhibit the carboxylase, suggested that there was little, if any, endogenous acyl-CoA present in the enzyme preparation.

TABLE 1. *Alleviation of palmityl-CoA inhibition of acetyl-CoA carboxylase by* DL-α-*glycerophosphate and by citrate*[a]

DL-α-Glycerophosphate or citrate addition (M)	With α-glycerophosphate			With citrate		
	$^{14}CO_2$ incorporated (mμmoles)		Inhibition (%)	$^{14}CO_2$ incorporated (mμmoles)		Inhibition (%)
	No palmityl-CoA	12×10^{-6} M palmityl-CoA		No palmityl-CoA	12×10^{-6} M palmityl-CoA	
0	6.0	1.0	83	6.0	1.0	83
0.04	8.0	3.5	66	6.0	3.0	50
0.08	9.5	5.0	47	8.0	4.0	50
0.16	11.0	7.0	33	11.5	6.0	48
0.32	14.0	10.5	25	19.0	10.0	47
0.60	16.0	14.5	9	24.5	16.5	33
1.60	22.5	20.0	11	28.0	21.0	25

[a] Each sample contained, in a final volume of 0.5 ml, the following: 0 to 35% ammonium sulfate fraction containing 0.27 mg of protein, 20 μmoles of potassium phosphate buffer (*p*H 7.0), 2.5 μmoles of manganese chloride, 5 μmoles of ATP, 0.5 μmole of acyl-CoA, 20 μmoles of $KH^{14}CO_3$ (10^6 counts per min per μmole), plus the additions indicated. Samples were preincubated for 15 min at 30 C with all reagents except α-glycerophosphate, citrate, and bicarbonate. α-Glycerophosphate and citrate were added to the appropriate tubes, and then all samples were preincubated for another 15 min at 30 C. The reaction was then started by the addition of bicarbonate and incubated for 30 min at 30 C.

DISCUSSION

As has been found to be the case in animal systems (e.g., 2), we illustrated that the acetyl-CoA carboxylase activity of preparations of this strain of *S. cerevisiae* was inhibited by palmityl-CoA (Fig. 1). This inhibition was relieved by the addition of α-glycerophosphate (Table 1), indicating that α-glycerophosphate stimulates acetyl-CoA carboxylase activity by removing inhibitory palmityl-CoA via glyceride synthesis. However, yeast preparations in which the acetyl-CoA carboxylase activity was strongly stimulated by the addition of α-glycerophosphate lacked detectable amounts of acyl-CoA compounds (Fig. 2). It might be maintained that inhibitory quantities of palmityl-CoA or long-chain acyl-CoA could be formed during the carboxylase assay. (Since acetyl-CoA and ATP are supplied, and malonyl-CoA is produced, traces of reduced nicotinamide adenine dinucleotide phosphate and fatty acid synthetase in the preparation might catalyze the formation of long-chain acyl-CoA compounds.) In other experiments (not reported here), this possibility was tested by using ^{14}C-acetyl-CoA in the carboxylase assay and then isolating the total lipids produced. Under these conditions, quantities of lipids several orders of

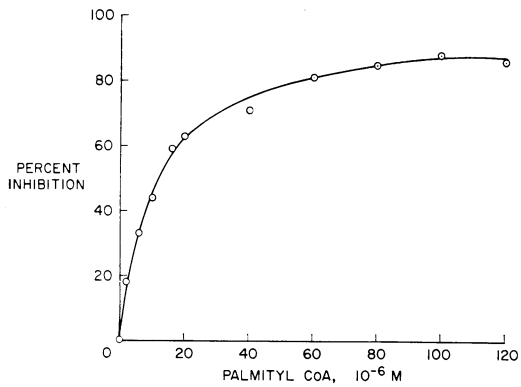

FIG. 1. *Effect of palmityl-CoA on the acetyl-CoA carboxylase reaction. Contents of the samples were the same as given in Table 1. Samples were preincubated for 15 min at 30 C with all reagents except bicarbonate. The reaction was then started by the addition of bicarbonate and incubated for 30 min at 30 C.*

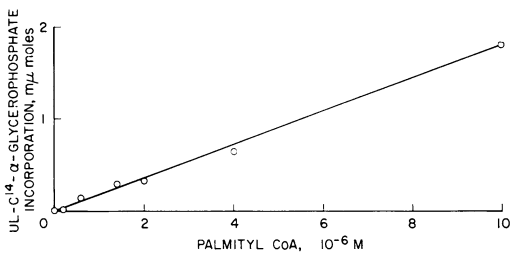

FIG. 2. *Effect of palmityl-CoA on glyceride formation. Each sample contained, in a final volume of 0.5 ml, the following: 0 to 35% ammonium sulfate fraction containing 0.26 mg of protein, 20 μmoles of potassium phosphate buffer (pH 7.0), 5 μmoles of ATP, and 0.1 μmole of uniformly labeled ^{14}C-α-glycerophosphate (4 × 10^5 counts per min per μmole). Samples were incubated for 45 min at 30 C.*

magnitude below that capable of exerting any inhibitory effect in the carboxylase assay were obtained, i.e., considerably less than millimicromole quantities of lipid material, calculated as palmityl-CoA.

These results do not preclude the possibility that palmityl-CoA, when present in appreciable amounts, would combine with α-glycerophosphate, and thus relieve palmityl-CoA inhibition. But since acyl-CoA was deficient in the preparations we used, our data suggest that α-glycerophosphate also stimulates acetyl-CoA carboxylase activity by some other mechanism, possibly an allosteric one. Indeed, α-glycerophosphate and citrate may affect the enzyme by a common mechanism.

Another finding which supports the existence of a common mechanism of activation of acetyl-CoA carboxylase by α-glycerophosphate and citrate involves the sequential addition of α-glycerophosphate and citrate. When maximally stimulating amounts of α-glycerophosphate were added to enzyme preparations previously incubated with maximally stimulating amounts of citrate, further stimulation of enzyme activity did not occur. Conversely, preparations first exposed to citrate and then to α-glycerophosphate showed no further stimulation. If α-glycerophosphate stimulated only by acyl-CoA removal, and citrate by some other mechanism, one would expect the stimulations to be additive.

We noted that citrate also relieved the inhibition by palmityl-CoA (Table 1), although it is not known to combine with palmityl-CoA in any enzymatic reaction; thus, this phenomenon supports the contention that α-glycerophosphate and citrate stimulate by a common mechanism. Density gradient experiments (11) suggest that neither citrate nor α-glycerophosphate stimulation involves a change in molecular weight, as has been observed in animal systems.

It has been reported that in the rat liver carboxylase system palmityl-CoA and citrate compete for the same allosteric site on the enzyme (10). The results given in Table 1 suggest the possibility that in the yeast system both α-glycerophosphate and citrate can compete with palmityl-CoA for sites that regulate the activity of the enzyme.

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